#9 Declaration Extended 7/10/01

CERTIFICATE OF MAILING
I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST-CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231, ON JUNE 28, 2001

Marianne H Michel
AGENT/ATTORNEY FOR APPLICANT

June 28, 2001
DATE

Attorney Docket No. 1109

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tao et al.	Date:	June 28, 2001
Serial No.:	09/496,444	Group Art Unit:	1638
Filed:	February 2, 2000	Examiner:	C. Collins
For:	"Cell Cycle Polynucleotides, Polypeptides and Uses Thereof"		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF WILLIAM J. GORDON-KAMM UNDER 37 C.F.R. 1.132

I, William J. Gordon-Kamm, declare:

I am a citizen of the United States of America, residing at 3916 67th Street, Urbandale, IA, 50322. I am employed by the applicant as a Research Manager.

I am an inventor of the above-identified application. I hold a Ph.D. degree and have approximately 16 years of experience in the transgenic plant production field. My curriculum vitae is attached at APPENDIX 1.

Both functional and structural distinctions are used to classify mammalian cyclins. These same criteria can be used to verify that the present inventive polynucleotides and proteins are Cyclin-E polynucleotides and proteins.

Cyclin A, B, D, and E proteins contain a highly conserved "cyclin box". As seen in Figure 1, there is strong homology in the cyclin box for Cyclin A, B, D and E

proteins from a variety of species (Zm = maize; Nt = tobacco; At = Arabidopsis; Mm = mouse; Hs = human; Dm = fruit fly). Within the cyclin box the maize CycE protein appears to be most closely related to both CycE's and CycB's from other species (Reference 1). Through high homology in the cyclin box we concluded that the protein is a cyclin protein.

Function of G1 cyclins (Cyclin D and E) is demonstrated though replacement or complementation of mutant yeast strains. Thus, cloning of a non-yeast cyclin gene into a yeast expression cassette and transformation of the G1-cyclin-deficient yeast strain will demonstrate whether the non-yeast cyclin gene functions in promoting G1/S transition. Cyclin A & B are mitotic cyclins and will not complement G1-cyclin-deficient yeast. This assay has been used to verify function (stimulating progression from G1 to S phase) for cyclin-D genes from various mammalian species, Drosophila, Arabidopsis, tobacco and other plants including maize (Figure 2). Likewise, Cyclin E genes from mammalian cells and Drosophila are known to stimulate this cell cycle transition and permit growth in the mutant yeast strain (Figure 2). As with the maize CycD, our putative CycE gene complements the G1-cyclin-deficient yeast. This functionally verifies that this gene is a G1-cyclin (Cyclin D or E) (References 2 and 3).

Two structural characteristics can be used to differentiate Cyclin E from Cyclin D. The first, unique amongst these four cyclins, CycD proteins across species contain the highly conserved Rb-binding motif, LxCxE, shown for maize, Arabidopsis and humans in Figure 3 (Reference 4). Cyclin E does not contain the Rb-binding box as can also be seen in Figure 3.

Secondly, amongst these four cyclins only Cyclin E proteins contain a CDK2 phosphorylation site (TPPxS) in the carboxy-terminus (Figure 4). This phosphorylation site is known to be involved in ubiquitin-mediated phosphorylation, earmarking these proteins for rapid degradation at the end of S-phase (References 5

and 6). This site is a well-characterized functional motif in Cyclin E proteins and is diagnostic of Cyclin E.

The combined evidence presented above indicates that the polynucleotides we have isolated encode a G1/S cyclin, and further that the combination of functional motifs present in the encoded protein distinguish this as a plant Cyclin E.

In summary the presence of a cyclin box indicates that the inventive proteins are cyclins. The complementation of the present polynucleotides in mutant yeast strains deficient in G1 cyclin indicates that the present polynucleotides encode a Cyclin D or Cyclin E protein. The lack of an Rb-binding motif indicates that the cyclin is a Cyclin E protein and not a Cyclin D protein. The presence of a CDK2 phosphorylation site further indicates that the protein is a Cyclin E protein.

References

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2. Lew,D.J., Dulic,V. and Reed,I.S. (1991) Isolation of three novel human cyclins by rescue of G1 cyclin (CLN) function in yeast. Cell, 66, 1197-1206.

3. Dahl,M., Meskiene,I., Bögre,L., Ha,D.T.C., Swoboda,I., Hubmann,R., Hirt,H., Heberle-Bors,E. (1995) The D-type alfalfa cyclin gene cycMs4 complements G1 cyclin-deficient yeast and is induced in the G1 phase of the cell cycle. Plant Cell, 7, 1847-1857.

4. Renaudin,J-P., Doonan,J.H., Freeman,D., Hashimoto,J., Hirt,H., Inzé,D., Jacobs,T., Kouchi,H., Rouzé,P., Sauter,M., Savoure,A., Sorrell,D.A., Sundaresan,V. and Murray,J.A.H. (1996) Plant cyclins: a unified nomenclature for plant A-, B- and D-type cyclins based on sequence organisation. Plant Mol Biol., 32, 1003-1018.

5. Won, K-A and Reed, S.I. (1996) Activation of Cyclin E/CDK2 is coupled to site-specific autophosphorylation and ubiquitin-dependent degradation of cyclin E. The EMBO J. 15, 4182-4193.

6. Gudas,J.M., Payton,M., Thukral,S., Chen,E., Bass,M., Robinson,M.O. and Coats,S. (1999) Cyclin E2, a novel G1 cyclin that binds Cdk2 and is aberrantly expressed in human cancers. Mol. Cell. Biol., 19, 612-622.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

William J. Gordon-Kamm

6/27/01
Date

Appendix A

WILLIAM J. GORDON-KAMM
Pioneer Hi-Bred International, Inc.
PO Box 1004, 7300 62nd Ave
Johnston, IA 50131

Education

Cornell University - Plant Science Department
Ph. D. Jan. 1985
Major: Plant Physiology
Minors: Plant Biochemistry, Plant Cell Biology

Western Washington University - Biology Department
M.S. Jun. 1980
Major: Botany

WWU – Biology Department; Bachelor of Science, Jan. 1979

Pertinent Employers

Pioneer Hi-Bred International, Inc., Johnton, IA
Position: Research Manager, Quality Traits Trans. Res., Jun 1, 1996 - present.

Pioneer Hi-Bred International, Inc., Johnton, IA
Position: Coordinator, Maize Elite Transformation, Nov. 1, 1994 - Jun 1, 1996.

DeKalb Plant Genetics, Mystic, CT 06355
Position: Senior Research Scientist, Feb. 1991 to Oct. 31, 1994.

DeKalb Plant Genetics, Mystic, CT 06355
Position: Research Scientist, May 1987 to Feb. 1991

New Mexico Highlands Univ., Division of Science and Mathematics, Las Vegas, NM
Position: Asst. Professor, Aug. 1985 to May 1987

USDA/ARS, Cereal Rust Laboratory, Univ. of Minnesota, St. Paul, MN 55108
Position: Visiting Scientist, May to Aug. 1986

USDA/ARS, Cereal Rust Laboratory, Univ. of Minnesota, St. Paul, MN 55108
Position: Preferred Post-Doctoral Associate, Oct. 1985 to Jul. 1985

Professional Associations

Soc. for In Vitro biology, American Society of Plant Physiology, American Society for the Advancement of Science, Society for Cryobiology, Sigma Xi

Current Research

The development of new transformation methods pulls together aspects of molecular biology and developmental biology. Past developments in this area have taken advantage of available (or modified) technologies in gene delivery and corn tissue culture, without a clear understanding of why these methods worked (and more importantly, why the methods didn't work in all corn varieties).

Many new tools exist that can help us dissect out pieces of this puzzle, answer basic questions (i.e. about gene delivery, integration, transgene fate and expression), and reassemble this information as we develop totally new, improved methods for genetic engineering. Of particular importance is improving our understanding of many of the underlying tenets in maize developmental biology. The creative use of transgenic plants directed towards questions about maize growth and development will be increasingly important towards this goal; examples include aspects of cell cycle control, hormonal signal transduction, homeotic regulation of plant development, embryogeny and fate development.

Teaching Experience

While at New Mexico Highlands University, I taught the following courses; General Biology I (organismal) and II (cellular), Genetics, Transmission and Scanning Electron Microscopy, and Molecular Genetics. I also conducted Special Topics courses in Genetic Engineering and Membrane Dynamics/Energetics.

Issued Patents

US5550318. Methods and composition for the production of stably transformed, fertile monocot plants and cells thereof. DeKalb Genetics Corp. (see also; EP0485506 & WO9102071)

US5489520. Process of producing fertile transgenic zea mays plants and progeny comprising a gene encoding phosphinothricin acetyl transferase. DeKalb Genetics Corp.

US5874265. Methods and composition for the production of stably transformed fertile monocot plants and cells thereof. DeKalb Genetics Corp. (see also; EP0721509 & WO95506128)

US5736369. Method for producing transgenic cereal plants (meristem transformation). Pioneer Hi-Bred International, Inc. (see also; EP0772687 & WO 9604392)

US5780709. Transgenic maize with increased mannitol content. DeKalb Genetics Corp. (see also; EP0889967 & WO 9726365)

US5929301. Nucleic acid sequence encoding FLP recombinase. Pioneer Hi-Bred Int'l. Inc.

Publications; see attached sheet.

WILLIAM J. GORDON-KAMM
PUBLICATIONS

Journal Articles:

LAURIE, J.D., G. ZHANG, L.E. MCGANN, W.J. GORDON-KAMM and D.D CASS. 1999. A novel technique for the isolation of embryo sacs from maize and the subsequent regeneration of plants. In Vitro Cellular & Developmental Biology 35:320-325.

SUN, Y., B.P. DILKES, C. ZHANG, R.A. DANTE, N.P. CARNEIRO, K.L. LOWE, R. JUNG, W.J. GORDON-KAMM and B.A. LARKINS. 1999. Characterization of maize (*Zea mays* L.) Wee1 and its activity in developing endosperm. Proc. Natl. Acad. Sci USA 96:4180-4185.

LOWE, K., BOWEN, B., HOERSTER, G., ROSS, M., BOND, D., PIERCE, D. and B. GORDON-KAMM. 1995. Germline Transformation of maize following manipulation of chimeric shoot meristems. Bio/Technology 13:677-682.

KAUSCH, A.P., ADAMS, T.R., MANGANO, M., ZACHWIEJA, S.J., GORDON-KAMM, W., DAINES, R., WILLETTS, N.G., CHAMBERS, S.A., ADAMS, W. WILLIAMS, G. and G. HAINES. 1995. Effects of microprojectile bombardment on embryogenic suspension cell cultures of maize (Zea mays L.) used for genetic transformation. Planta 196: 501-509.

GORDON-KAMM, W.J., SPENCER, T.M., O'BREIN, J.V., START, W.G., DAINES, R.J, ADAMS T.R., MANGANO, M.L., CHAMBERS, S.A., ZACHWIEJA, S.J., WILLETTS, N.G., ADAMS, W.R, MACKEY, C.J., KRUEGER, R.W., KAUSCH, A.P. and P.G. LEMAUX. 1991. Transformation of maize using microprojectile bombardment: an update and perspective. In Vitro Cell. Dev. 27P:21-27.

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STEPONKUS, P.L. and W.J GORDON-KAMM. 1985. Cryoinjury of isolated protoplasts: A consequence of dehydration or the fraction of the suspending medium that is frozen? Cryo-Lett. 6:217-226.

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STEPONKUS, P.L.,DOWGERT, M.F. and W.J GORDON-KAMM. 1983. Destabilization of the plasma membrane of isolated protoplasts during a freeze-thaw cycle. The influence of cold acclimation. Cryobiology 20:448-465.

Chapters in Books:

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MACKEY, C.J., SPENCER, T.M., ADAMS, T.R., LEMAUX, P.G., KAUSCH, A.P., KREUGER, R.W. and W.J. GORDON-KAMM. 1993. Transgenic maize. IN: Transgenic Plants, Vol. 2, Present Status and Social and Economic Impacts, (S-D. Kung and R. Wu, eds.), Acad. Press, Inc., San Diego, pp. 21-31.

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STEPONKUS, P.L.,DOWGERT, M.F.,EVANS, R.Y. and W.J GORDON-KAMM. 1982. Freeze-induced dehydration: Effects on the plasma membrane of isolated protoplasts. IN: Biophysics of Water, (F. Franks, ed.), Wiley-Interscience, Chichester, pp. 319-322.

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LOWE, K., ROSS, M., BOWEN, B., HOERSTER, G., CHURCH, L., TAGLIANI, L., BOND, D. and GORDON-KAMM, W. 1996. The maize meristem as a transformation target. In Vitro Cellular & Dev. Biol. 32(3):31A.

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GORDON-KAMM, W.J., ADAMS, T.R., ADAMS, W.R., CHAMBERS, S.A., COURREGES, V.C., DAINES, R.J., MANGANO, M.L., O'BRIEN, J.V., SPENCER, T.M., START, W.G., WILLETTS, N.G., KAUSCH, A.P., KRUEGER, R.W., LEMAUX, P.G. and C.J. MACKEY. 1989. Stable transformation of embryogenic maize cultures by microprojectile bombardment. J. Cell. Biochem. Suppl. (Keystone Symposium)

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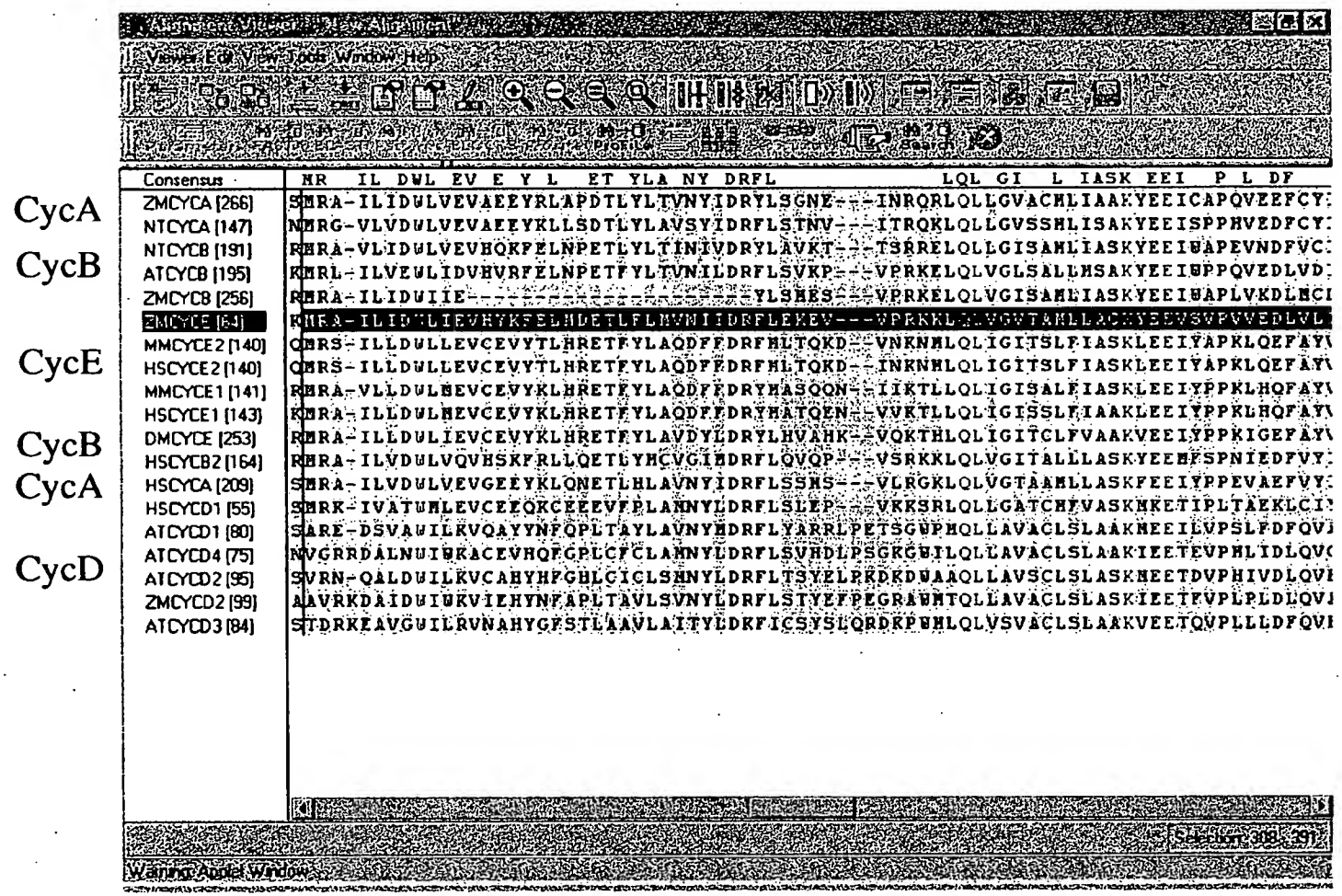
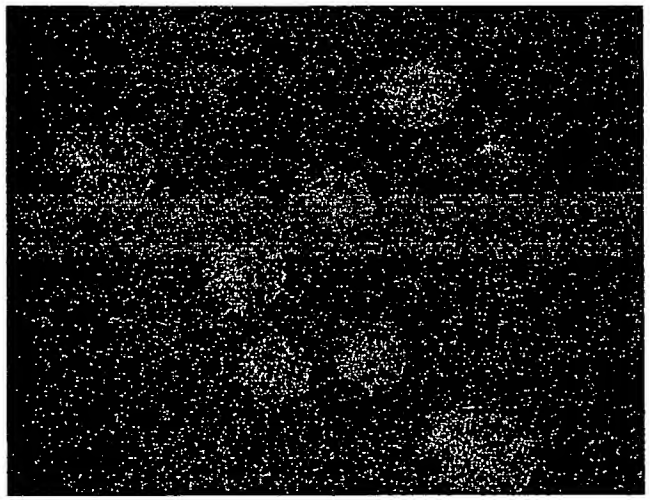
Figure 1. Conserved cyclin box among all cyclin proteins



The cyclin box in maize CycE protein is closer related to cyclin boxes in CycE and CycB proteins than to that in CycD proteins.

ZmCycD



ZmCycE

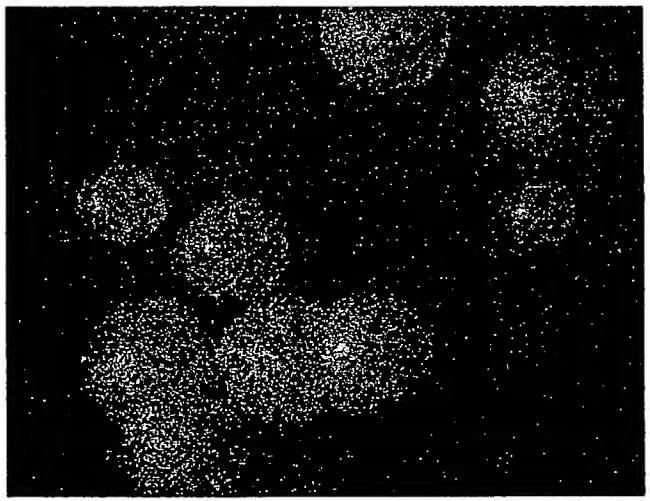
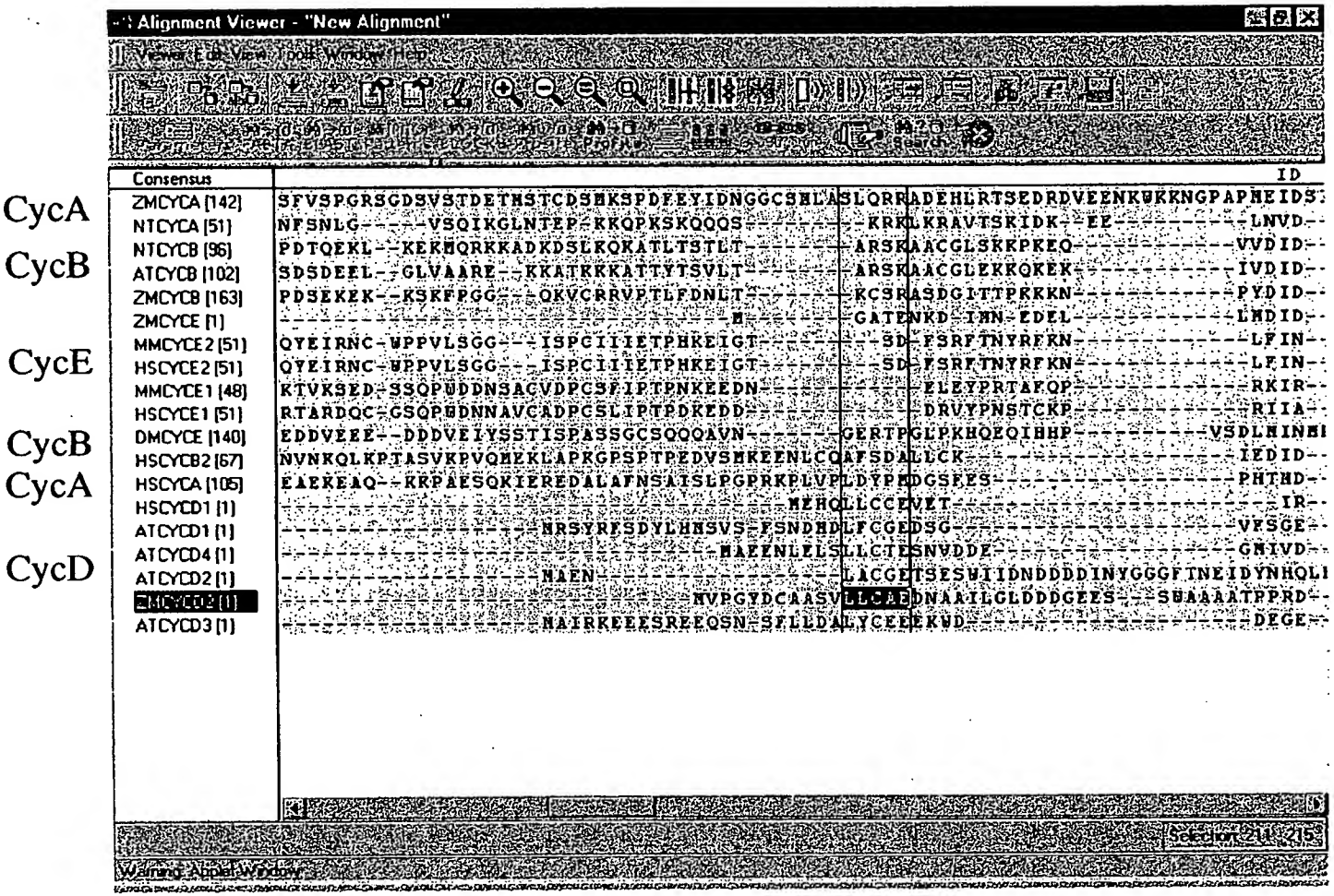


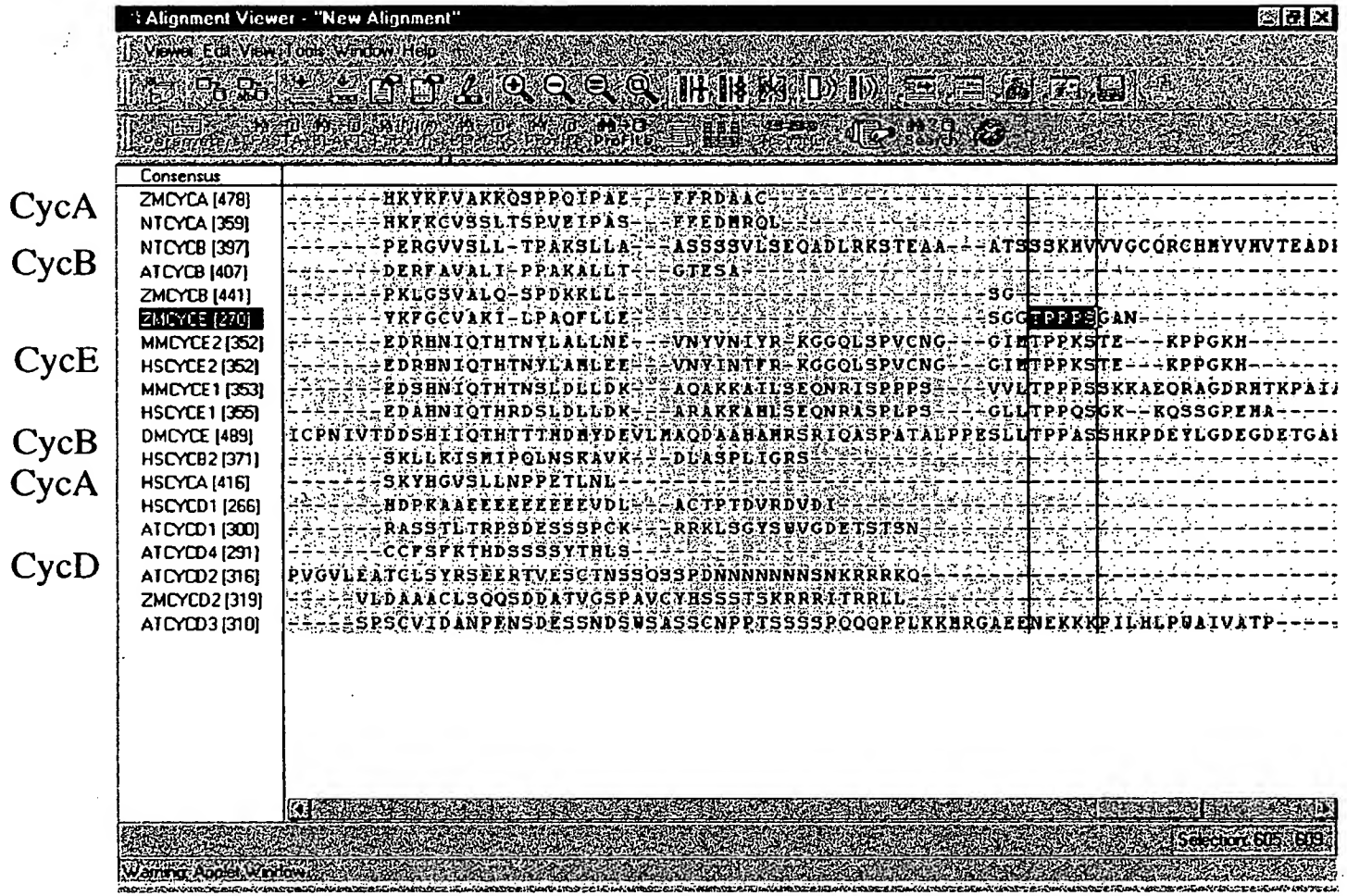
Figure 2. Complementation of a yeast strain defective G1-cyclins (cln1-, cln2-, cln3-).

Figure 3. Conserved Rb-binding motif in CycD proteins but not in other cyclins



Maize CycE does not contain a C-terminal Rb-binding motif.

Figure 4. Conserved CDK2 phosphorylation site in CycE proteins but not in other cyclins



Maize CycE protein contains a CDK2 phosphorylation site, TPPXS, in the C-terminal region.